Chapter 6

Optimization of Bone Scaffold Engineering for Load Bearing Applications

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Summary

This review highlights concepts that are required for successful generation of bone scaffolds to be used in load bearing applications. Through a discussion of the bone hierarchical levels, scaffold fabrication methods, and bone modeling response, a paradigm shift in the basic set of rules for scaffold generation is illustrated. Following these suggested guidelines may lead to the design of scaffolds that closely emulates the overall properties of bone and may successfully heal bone defects in orthopaedics.

Keywords: Bone Hierarchy, Bone Scaffolds, Rapid Prototyping, Scaffold Engineering, Tissue Engineering

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Introduction

The intended use of bone scaffolds is for implantation in critical size bone defects, which in most cases need to sustain mechanical loading. Successful scaffold design should stimulate new bone growth resulting, at the end state, in native bone tissue with no trace of the scaffold. The desire to accomplish this task and the current state of the field are far from meeting. Current designs have failed to produce a scaffold that is able to remain viable throughout the duration from implantation to the end of the healing phase under load-bearing conditions, thereby missing the goal of an optimized and reordered bone architecture that is as functional and stable as the native bone. Only with the incorporation of both biomechanical considerations and biological requirements to produce a globally optimized structure from the overall shape to the chemical composition of the therapeutic device can there be hope for the success of both the implant and the subsequent ingrowth of bone. The first step in the healing of a bone defect begins with the implantation of the engineered scaffold. Following implantation, the scaffold takes the entire load of the defective region and the surrounding area is subjected to the normal state of stress. Bone modeling (also known as bone adaptation) begins at this point as a result of mechanosensing of the bone and degradation of the implant. At the end state of this process, after degradation of the implant, none of the scaffold material remains and newly formed bone occupies the site previously taken up by the scaffold. At this endpoint, the bone once again carries the entire load applied to the tissue and is in a state of stress relative to the pre-defect condition. During the time course starting at implantation and leading to native bone tissue, little is known of the interaction between the scaffold and the bone. Additionally, any control over the healing response is lost upon implantation of the scaffold into the defect. Following implantation, any surgical intervention to possibly re-engineer any treatment is not desirable due to the invasiveness of the procedure and the limited access to the scaffold. At that point, the bone modeling process takes over, modifying the design and mechanical characteristics of the scaffold on its own.

General Considerations

The success of a bone scaffold as measured *in vivo* is determined by its ability to stimulate and aid in both the onset and completion of bone defect repair. Because the only control parameters that can be affected prior to implantation are the incorporation of growth factors, cell seeding and architecture modification, optimization of the scaffold must be completed prior to use and must encompass at the very least mechanical stability for its load bearing application. This optimization requires a complete knowledge of the system the scaffold will be interacting with. Previous studies have taken steps towards characterizing the mechanical environment existing in load bearing sites of various animals. Measurements with strain gauges on long bones of horses, sheep, pigs, and humans have allowed the calculation of the average strain during normal loading conditions, such as walking or running. Due to animal size, bone cross sectional area, and body weight, the stress levels on each bone vary greatly between animals but regardless of species, average strain is similar (1). Similar strain rates regardless of stress levels or concentrations indicate that remodeling characteristics of bone are similar throughout the animal kingdom. It should be noted that bone adaptation and resorption rate has been shown to differ between species, especially when compared between fast and slow growing animals. This knowledge allows the prediction of desired mechanical characteristics for bone scaffolds based on similar bone modeling steps. If we take a look at bone tissue of one species (i.e. humans) in particular, we generally differentiate only between trabecular and cortical bone. Previous studies on the mechanical properties of bone tissue, however, have revealed that trabecular bone material properties vary significantly between anatomic sites (2, 3). Nevertheless, it was found that the tissue properties of trabecular and cortical bone from different anatomical sites are comparable. We can therefore draw another conclusion in that the reorganization of the structure of a material may lead to a globally optimized structure supporting a variability of several magnitudes of stress levels.

The loading conditions of bone stay within a specific range during normal actions, such as walking (1). This indicates a dual need for the function of an implant, one which changes halfway through the treatment. At the time of implantation, the scaffold must serve as a load bearing device and provide mechanical stability. Following implantation, the load must be completely transferred to the bone and the scaffold must serve as a therapeutic material. Allowing ingrowth by high progression

of scaffold degradation and subsequent strength, the load is transferred gradually to the bone stimulating bone healing. Scaffolds must be tailored to incorporate more than just the adequate stress concentrations in order to sustain the load and remodeling of an area of bone. Complete design of an implant must take into account both the mechanical considerations and the biological consequences of the implant site. This review illustrates ground rules for the optimization of scaffolds based upon various design requirements to create a bone scaffold for load bearing applications. Through understanding the levels of bone hierarchy, scaffold fabrication methods, and bone remodeling response, a fully optimized bone scaffold can be created.

Scaffold Design

An optimized scaffold design must incorporate the elements of both biological and mechanical characteristics of the studied system. Current scaffold design is centered on the perfection of either the biological constraints or the mechanical requirements of the defect site. The biological environment of bone contains the fluid and nutrient transfer as well as the cell types that are contained in bone; a scaffold tailored to this may be efficacious in stimulating cell migration and matrix deposition. The mechanical environment of bone involves the loading requirements as well as the spatial localization of bone cell types to promote cell-cell signaling; scaffolds engineered for this environment may be successful at transferring mechanical loads. Failure to incorporate both of these design criteria into the entire package will inhibit the success and longevity of treatment. Bone, like any biological system is a summation of its components and these components or phases can be evaluated in a hierarchical structure. Bone is a composite material that exists on at least 5 hierarchical levels: whole bone, architecture, tissue, lamellar, and ultrastructure (Fig. 1).

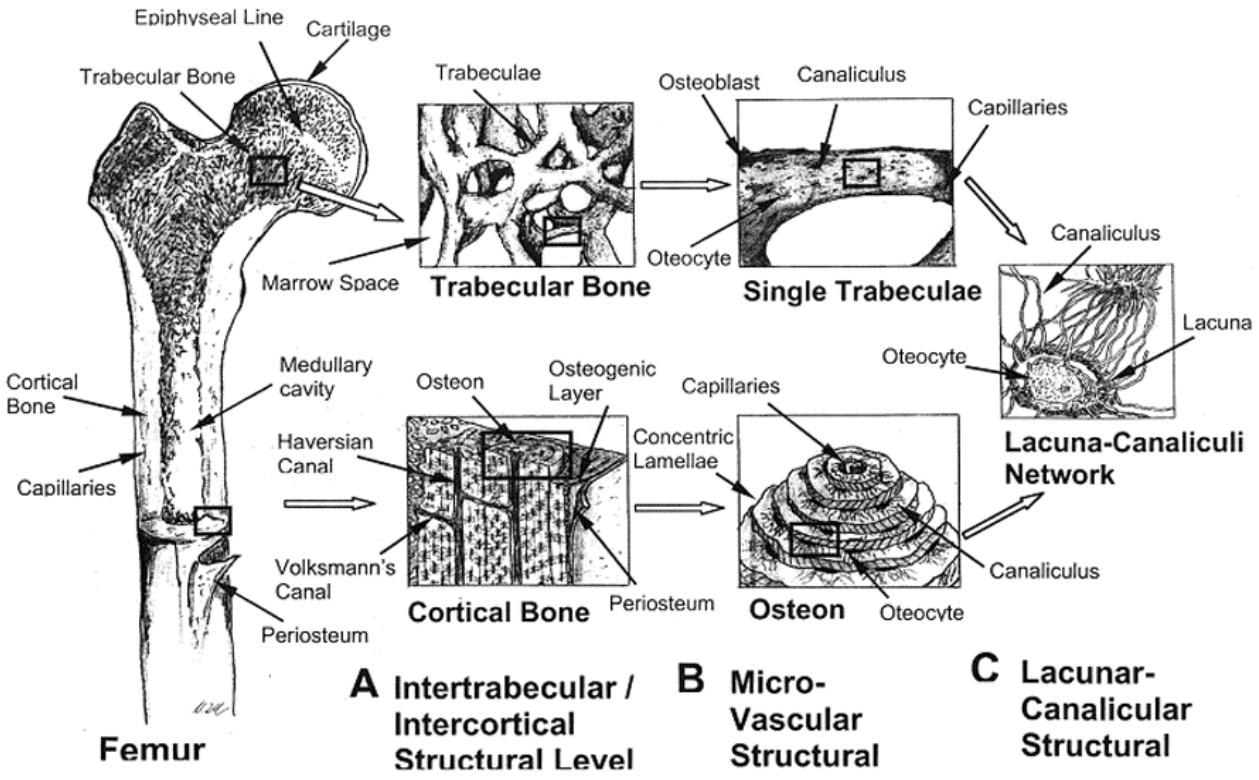

Fig. 1: Bone Hierarchy

The whole bone level is the top level and represents the overall shape of the bone or scaffold. This structure is composed of the architectural level, which contains the microstructure that defines the spatial distribution. Below the architectural level is the tissue level, which is inherent to the actual material properties of bone. The lamellar level is below the tissue level and is composed of the sheets of collagen and minerals deposited by osteoblasts (4). The final level is the ultrastructural level which incorporates chemical and quantum interactions (5). These levels (6) comprise structural differences between magnitudes of size between the subsequent levels, spanning from the whole bone to the chemical and quantum level. In order to expedite the analysis of bone and its constituents, each separate constituent that contributes to the system as a whole must be evaluated. There are certain advantages that can be gained by separating the structure into microstructural organizational levels. At the hierarchical level, it is easy to compare different structures and tissues.

Additionally, it is much simpler to define characteristic levels to use for analysis. Each level depends on the lower levels to provide function and structural support for the top levels (Table 1).

Level	***Dimensions***
Whole Bone Level	3mm - 750 mm
Architectural Level	75 – 200 μm (T) 100 – 300 μm (C)
Tissue Level	20 – 75 μm (T) 20 - 100 μm (C)
Lamellar Level	1 – 20 μm (T) 3 – 20 μm (C)
Ultrastructural Level	.06 - .4 μm (T) .06 - .6 μm (C)
T = Trabecular Bone; C = Cortical Bone	

Table 1. Bone Hierarchy Levels (6, 15)

a) Whole Bone Level

The top level of bone is the organ level or whole bone level and it is the result of the summation of all of the lower levels of bone. At this level, the bone functions on the order of magnitude of the organism, providing structural support and aiding i.e. with locomotion. The mechanical characteristics of whole bone are a result of the geometry of the whole structure (Fig. 2). At this hierarchical level, the bone may interact with other bones, joints, or muscles in the body. Optimization at this level is as a result of the need of the organism for strength in the whole bone, and not as a result of localized stress concentrations. Shape changes that occur at this level are minimal and the mechanical strength of the structure is a result of the total geometry of the bone and

the distribution of the tissue. Remodeling that may occur at lower levels is measured as percent increase or decrease in mass in the overall bone (7).

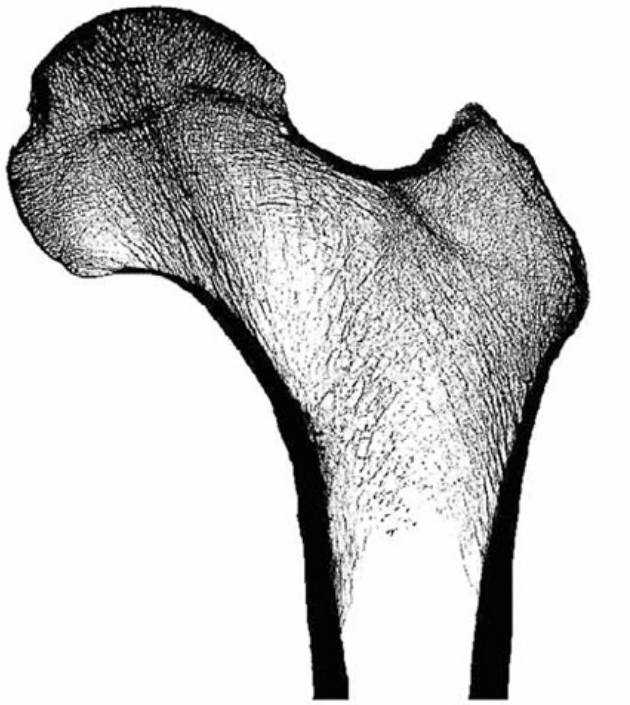

Fig. 2: Example of whole bone level – Human femur

b) Architectural Level

The architectural level of bone relates to the characteristic micro-architecture of bone tissue, specifically cortical or trabecular bone. One step below the global structure is the architecture which serves to provide mechanical stability to the entire global structure of bone. The optimized architecture of bone shares the overall load of the entire organ and is distributed throughout the osteons and/or trabeculae. It is at this level that the effects of remodeling are seen as a change in geometry or architecture and in the apparent mechanical properties. Depending on the type of bone, trabecular or cortical, two different architectures will arise. Trabecular bone, contained in the end of long bones and the site of bone marrow synthesis, exhibits anisotropy as a result of its rod and plate

organization. Cortical bone is highly compact and orthotropic due to the circular nature of the osteons that make up its structure. One illustration of the micro-architectural differences between the two architectures is that cortical bone contains only microscopic channels through the center of the osteons whereas trabecular bone is highly porous (Fig. 3 & 4) (8).

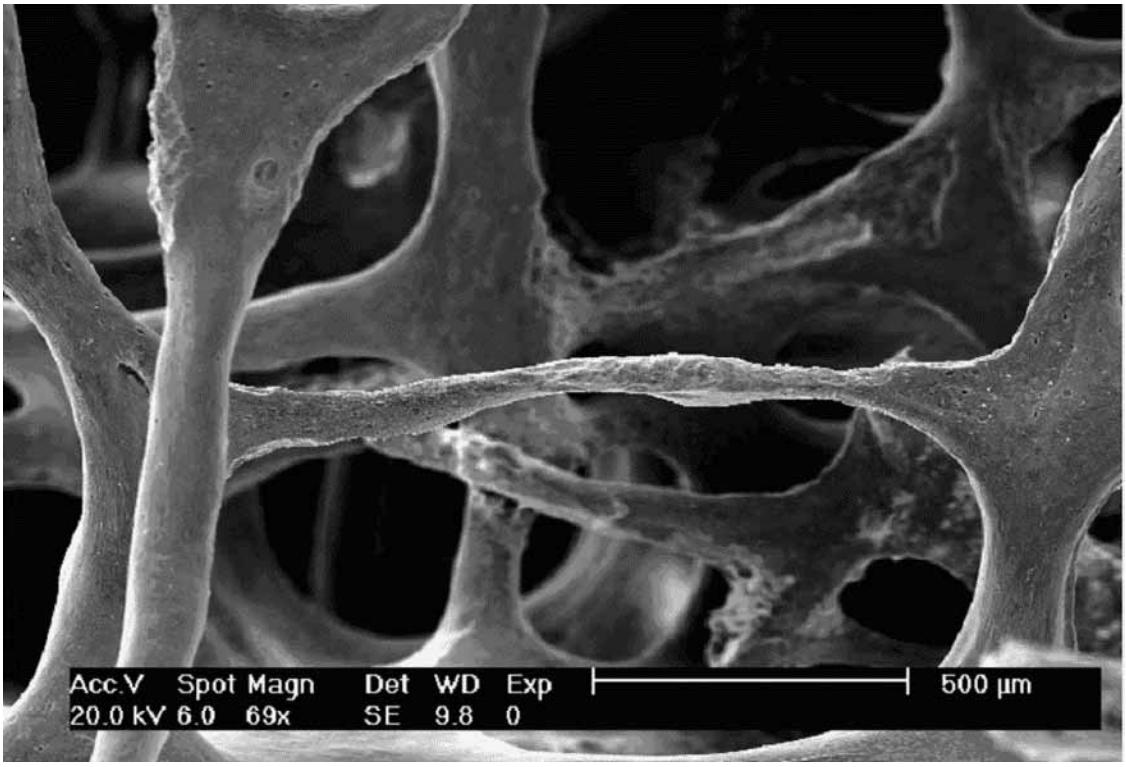

Fig. 3: Trabecular bone (50)

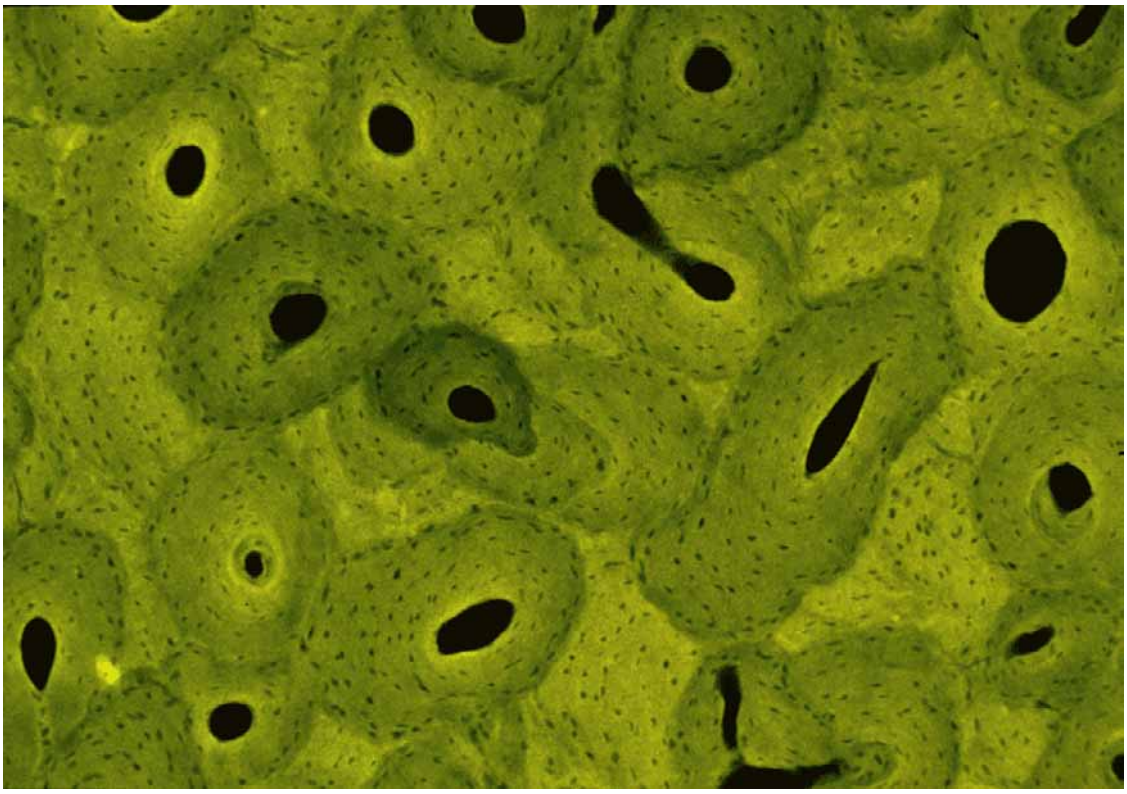

Fig. 4: Cortical bone (15)

Mechanical function at the architectural level is to provide support for the overall bone structure and, specifically in trabecular bone, as a shock absorber and to resist compressive loads (9). The mechanical strength can be related to several geometric constraints such as trabecular thickness, density, and bone surface to bone volume ratio, which can be obtained from imaging techniques used to evaluate trabecular tissue (10). Strain sensed in the bones at this architectural level causes the cells on a lower hierarchical level to remodel the gross arrangement of the micro-architecture only on the surface (11). Although gross reorganization of the bone micro-architecture is seen at this level as a change in geometry and architecture, the deposition and resorption occurs at the cellular level. Orientation and mechanical qualities change between anatomical sites and between bones as a result of dynamic loading and stress upon the bone tissue. The advantage of addressing bone at this level is that the sequential architectural structures can be viewed as a continuum. The use of continuum mechanics aids in the analysis of predicted stress and strain and can simplify analysis of stress concentrations. The mechanical characteristics of the architectural level are largely due to the spatial

distribution of the tissue (micro-architecture) and less so due to the properties of the material composing bone.

c) Tissue Level

Below the architectural level of bone is the tissue level, which directly addresses the mechanical properties of the tissue. The material properties at this level provide support for the geometry of the architectural level above it. Remodeling of bone at this stage of the hierarchy alters the material properties of the bone tissue. The tissue properties are those that relate directly to the mechanical characteristics of the bone independent of the micro-architecture. Properties such as stiffness, Young's Modulus, yield point, and energy to fracture can be dealt with on a fundamental material level. The design of scaffolds at this level would allow the choice of material based on its mechanical properties rather than its architecture or ability to form a global structure. At this level, the material properties are what strengthen the architectural level of the bone.

Design of materials to be used in load bearing scaffolds has led to the improvement of biomaterials for implantation in the body. The problem with most of the materials is the failure to match the stiffness or strength of either trabecular or cortical bone (Table 2). This inability to match strength interrupts the first goal of the scaffold, which is to remove the mechanical loading from the bone defect site in order to reduce stress shielding. While the micro-architecture of the implant can be optimized for maximum strength and/or stiffness, the material choice is still one of the most important aspects of the treatment design. Depending on the material properties, some biomaterials are too weak to be arranged into the desired architecture and some materials are too stiff and would fracture when arranged into certain architectures (12, 13). Both the architectural level and the tissue level must be designed in concert to elicit both spatial distribution and a material that result in overall mechanical properties that are sufficient to sustain loading.

Material	***Strength (MPa)***	***Young's Modulus E (GPa)***
Cancellous Bone	2.23-7.36	67-445
Poly (lactic acid)	28 – 50	1.2 – 3
Bioglass-ceramics	500	22
Poly(methyl methacrylate) PMMA	30	2.2

Table 2. Some mechanical properties of biomaterials (3, 52)

d) Lamellar Level

Below the tissue level of bone is the lamellar level, the layers of bone deposited by single cells. Lone structures, the lamellae are laid on top of each other like composite board in directions that vary by up to 90 degrees. These laminations are the lowest form of bone and are deposited by the basic multicellular unit (BMU). This process involves a recruitment of osteoclasts that resorb bone, which is then followed shortly by osteoblast recruitment, which deposit bone. With the recruitment of the osteoblasts begins the deposition of new bone and ends with the osteoblasts becoming encapsulated in the bone matrix themselves and differentiating to mechanosensing osteocytes. The osteoblasts deposit a layer of hydroxyapatite onto a woven bed of collagen (8, 14). The sheets of lamellae are on the order of 3-20μm in thickness (15). It is this process that results in all of the lamellar bone (Fig. 5) in the body, which is a much stronger and better form of bone than embryonic or woven bone.

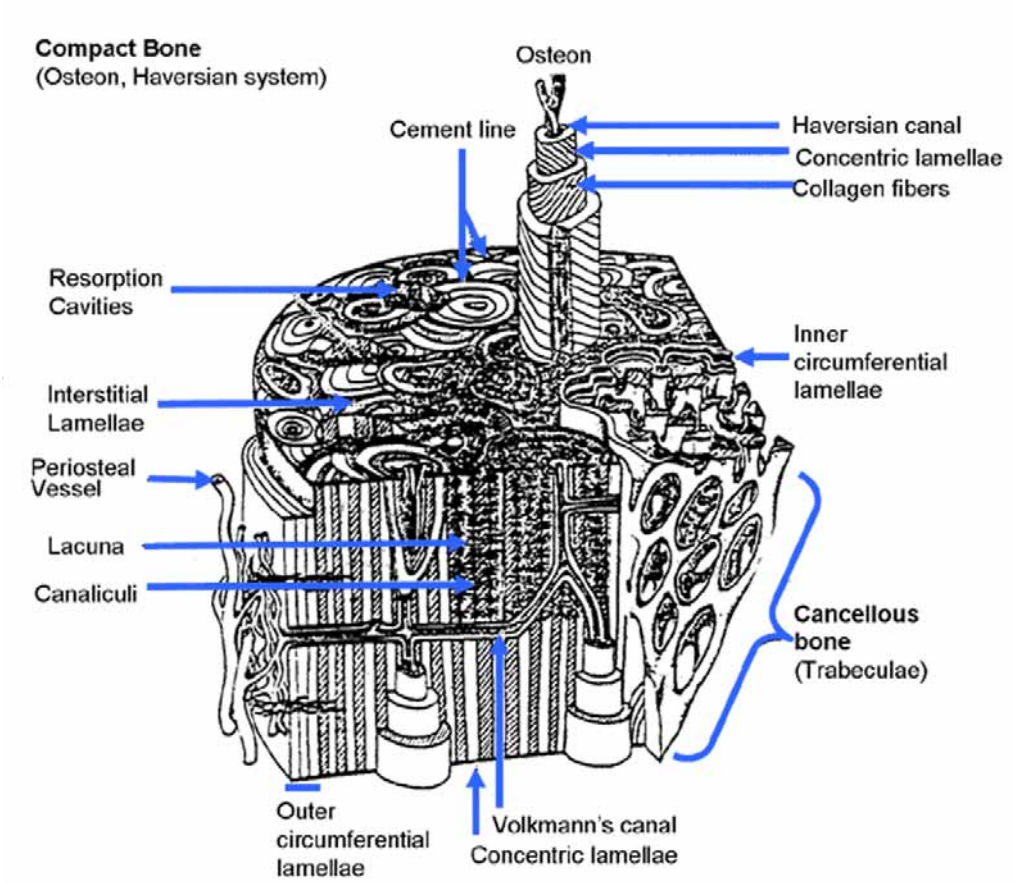

Fig. 5. Diagram of Lamellae (51)

The deposition and resorption of bone occurs only at the surface, however, and the lowest layers of lamellae are not affected unless massive bone loss is experienced, as in osteoporosis. Mimicry of this design would be similar to the micro-design of the thickness of one layer of common biomaterials used for some bone applications, poly(lactic-glycolic acid) (PLGA) or poly(propylene fumarate) (PPF) (14).

e) Ultrastructural Level

The lowest level of the bone hierarchy considered in this review is the ultrastructural level. At this level chemical and quantum effects can be addressed. The order of magnitude for this level allows the analysis of the mechanics and architecture of the collagen fibers with the minerals (5). This level is on the order of calcium and other minerals that are a part of bone, such as phosphate and magnesium (Table 3). The advantage of viewing bone at this level is that it incorporates an additional function of bone that cannot be addressed until this size, which is the use of bone as mineral storage for the organism. This mineral storage and the effects of chemistry are the main functional points at this level as is the orientation of collagen in the lamellae (4). The design of bone at this level illustrates how the micro-architecture of the structure must be evaluated as well as the nano-architecture. Several studies have been completed on the difference in mechanical properties as a result of the collagen orientation and the amount of mineral deposition on the collagen beds. The degree of mineralization will affect the final stiffness of the bone itself as well as the overall ash content (6). Design of an implant must include the evaluation of the chemical constituents, which will interact with the isotonic concentration of a fluid as well as with the chemical composition of the surrounding media and tissue in the implantation site. Additionally, an implant that is optimized at this level must address the chemical constituents of the polymers that will interact both with the cells and with the chemicals in the surrounding bone fluid.

Chemical Composition of Bone	***Percentage***
Calcium	26.7
Phosphorous	12.47
Carbonate	3.48
Sodium	0.731
Magnesium	0.436

Table 3. Composition of Major Chemicals in Bone (53)

Consideration of Bone Hierarchy into Design Principles

The use of Computed Tomography (CT) and other imaging techniques have aided in the design of the overall structure of therapeutic technologies. Success has been shown to aid those with the disorder of microtia, a congenital deformity of the external ear where the auricle (the external ear) of one ear may be severely deformed (16). By obtaining the three-dimensional volume of the good ear and building a model using solid freeform fabrication (SFF) it is possible to produce a silicon ear that can be used under a skin graft to repair a cosmetic defect [Unpublished data]. While the global structure of the ear is sufficient to repair the cosmetic function, all other orders below the structure level are not addressed. The silicon will exist in the body for extended periods of time without eliciting an immune response, but tissue ingrowth and remodeling will not occur. The functionality of the implant is based solely on the fact that the global shape is correct for its intended cosmetic purpose (Fig. 6 & 7).

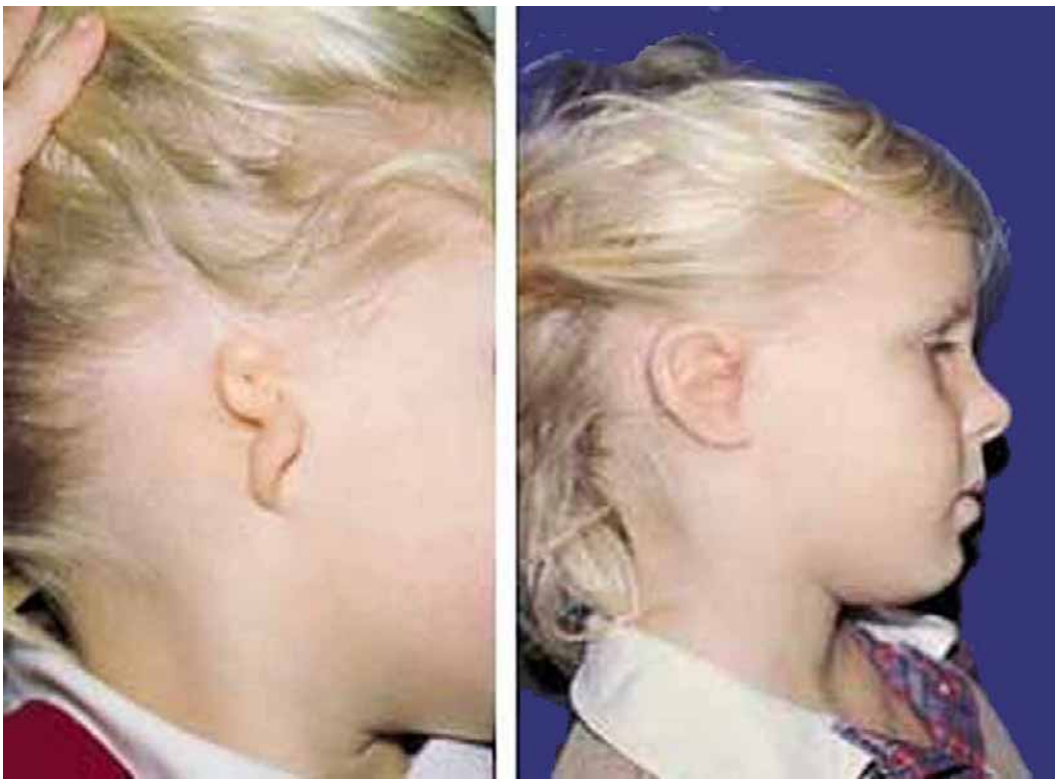

Fig. 6: Pre and post-operative patient with microtia (16)

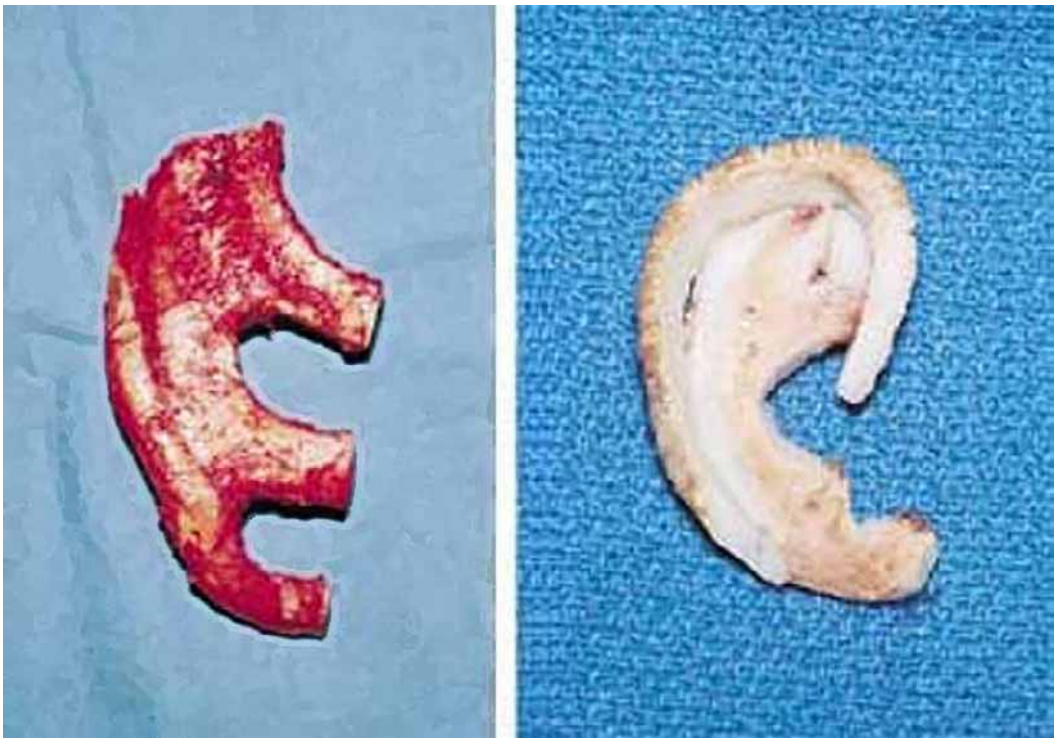

Fig. 7: Costal cartilage shaped into ear structure (16)

An additional example of the design of overall structure of the scaffold can be shown in yet another ear study. Using porcine chondrocytes and Yorkshire swine, a helical contour in the shape of an ear was fashioned. Skin of the pig was sutured in the shape of the ear and a hydrogel/chondrocyte mixture was injected into the hollow channel. The construct was allowed to grow for 10 weeks and was then removed from the pig's skin. The resulting shape exhibited the characteristics of an ear with high elasticity and vascularization throughout the tissue (17). This study represents the usage of several levels of hierarchy. The overall structure of the implant was shaped into the structure of the ear and the polymer and the cells regulated the internal architecture. The results of this study indicate that this technique could be used successfully to further the design of the ear.

The usage of Finite Element Analysis has shown great promise for the creation and optimization of bone scaffold architecture. Using images obtained from μCT of real bone, the approximation of the volume and the apparent properties can be transferred to a model with the same porosity and

trabecular thickness but with an optimized architecture. Many studies have attempted to accomplish this task with the creation of a scaffold that has a tailored architecture to promote the mechanical stability, porosity or stiffness of trabecular bone (18, 19). Many of these scaffolds employ an open-cell repeated architecture that contains orthogonal struts (Fig. 8) and porosity similar to bone (12, 20). While the scaffolds may be optimized for the overall structure and the architecture is similar to bone, failure to address lower hierarchical levels may inhibit the success of the implant. Mechanical strength of the implant is due also in part to the material that is used in the construction and several architectures that have been used in previous studies (21, 22).

Fig. 8. Composite orthogonal scaffold

Scaffolds that have been optimized for architecture deal mostly with the second level of bone, which is the architectural level. Hollister *et al.* have published much work detailing the development of scaffolds with engineered micro-architecture as a function of the desired or predicted stress concentrations existing in bone scaffolds (23). These scaffolds exhibit internal architecture that is regular throughout the structures with porosity values comparable to trabecular bone. The desired stress concentrations are similar to what is experienced *in vivo* regardless of the material used in the scaffold construct because the architectural and tissue level properties are responsible for the stress distribution and not the material levels. However, many materials lack the mechanical strength to support stress levels or architectures similar to the *in vivo* environment (24-26). Still, many other scaffolds have been designed for intended use in bone scaffold engineering that have a regulated, engineered internal architecture (22). These scaffolds are designed to be loaded and may be globally shaped into the desired shape. While these scaffolds are designed for load bearing applications, they fail to be designed for biological considerations such as cellular ingrowth. These scaffolds will only produce bone as a result of mechanotransduction by the osteoblasts, which may not even adhere to the scaffolds.

The final example deals with both the architectural and material levels of bone scaffold engineering. By taking CT images of a wound site and the contralateral side of healthy tissue, an approximation of the defect area can be obtained. By developing a finite element scheme, the mechanical requirements of the area can be determined. Areas of high and low stress concentration will manifest themselves allowing the determination of the areas that will require the most mechanical stability. By designing an implant that has strong and weaker areas, this stress gradient can be fulfilled. One research group has accomplished this by using architecture that mimics the desired mechanical stability and designing a global architecture as a result of this finite element analysis (FEA) study (19). The result is an optimized global shape that has architecture which is optimized for the desired mechanical requirements.

A study by Yang and associates (27) addressed the chemical elements of bone growth. They have incorporated a calcium agonist into the chemical composition of scaffolds made with a stereoregular polymer, poly(L-Lactic acid) (L-PLLA). By implanting the scaffolds in tissue the theory indicated that voltage operated calcium channels would stimulate bone growth. The results upon

implantation demonstrated that the incorporation of the calcium channel agonist served to increase the transduction of load in the scaffolds. In the long term, this may increase the effect of mechanotransduction, which could help to stimulate cells to both deposit bone and migrate into the scaffold. This technique is an adequate example of both the material and chemical level of bone tissue engineering. With an optimized architecture and proper global shape, an optimized scaffold can be created.

Few examples exist that can detail the incorporation of all of the hierarchical levels of bone. The previous examples for each level have served to indicate that it is necessary to evaluate and address all components of the desired effect of the healing agent in order to accomplish the clinical goal. Only with the complete top to bottom design of an implant can success be expected in the healing of bone defects in sites experiencing load bearing. Added to the fact that few (28-32), if any studies have created an implant that is both mechanically stable and addresses all design components, there exists little literature detailing the tests of scaffolds in a load bearing setting. The majority of bone growth studies have been accomplished in cranium models, however the total number of clinical cases with cranium defects is relatively low compared to other orthopaedic cases. For us to know if a scaffold will truly be successful in a load bearing application, we must test our work in such a setting. It is only after this testing that we can determine that the regrown bone is in fact quality bone and will be able to sustain the desired load. The lack of research that is being conducted in load bearing applications leads us to believe that the current designs for scaffolds are not fully taking into account the final target, which is to regenerate bone in a critical size bone defect that experiences mechanical loading.

Rapid Prototyping Methods for Scaffold Creation

New methods of scaffold fabrication are now able to attain the resolution of individual trabeculae. Combining technologies such as Solid Freeform Fabrication (SFF) with imaging techniques and computer modeling, tailored scaffolds can not only be designed but actually created to scale.

Architecture can now be laid out with regular, repeated structures, composed of almost any biomaterial that is desired (33). The previous designs have shown that it is impossible to implant an architecture that is the exact replica of trabecular bone because a structure such as that represents an end state, not a beginning such as needed for fixing a bone defect. Bone ingrowth occurs through scaffold surface deposition; once a solid bone surface is generated the scaffold degrades, leaving a hollow structure. However, the actual time line of this mechanism has not been investigated yet, only the results at the start and end points have been reported. Based on this theory the start geometry of the scaffold and the end geometry of the bone can never be the same. The failure of these previous bone micro-architecture replicating experiments indicates a need for a globally designed construct with internal architecture able to support load, degrade, and allow for new bone ingrowth. The geometric details that are required for such a design have only recently become possible to control and previous techniques could only achieve internal architecture using methods which generated random internal architecture. Creation of random architecture may produce a scaffold that has a desirable global structure but is lacking all other hierarchical levels. It is only with directed scaffold generation that an engineered scaffold with regulated internal architecture can be built.

Basic Scaffold Generation

The basic goal of the aforementioned manufacturing techniques is to produce micro-architecture in a scaffold that is highly porous to allow for cell adhesion, vascularization, and nutritient flow. Utilizing basic chemistry and/or the simple properties of the polymers, generation of scaffolds with non-specific methods results in scaffolds that are random with respect to several different parameters such as porosity, architecture, and anisotropy. Although these scaffolds are of a far inferior quality compared to the engineered scaffolds, simple protocols have fueled their use. The most often used methods to produce architecture are with solvent casting, gas elution, or melt molding (34, 35). Success in the stimulation of bone has been shown with scaffolds with random architecture and porosity similar to bone. The advantage of these methods is that they can be

combined with processes that generate global structure, and even with rapid prototyping to result in a scaffold optimized on the whole bone level with a random architecture at the tissue level.

Solvent casting involves the incorporation of solid particles, such as NaCl into a liquid polymer. Following gellation or casting of the polymer, the particles are removed using a solvent, usually water or Ethanol (36). The resulting global structure contains architecture that is a result of the random spatial organization of the particles in the polymer, with limited control of anisotropy and porosity range (Fig. 9). Porosity of the final solid can be adjusted based upon weight percent. In order to achieve an open cell architecture required for vascularization and nutrition flow, the porosity of these randomly organized scaffolds needs to be very high, more than 60% by volume (37). Sieving can also be used to obtain specific particle size resulting in a range of pore sizes in the final solid (35, 38).

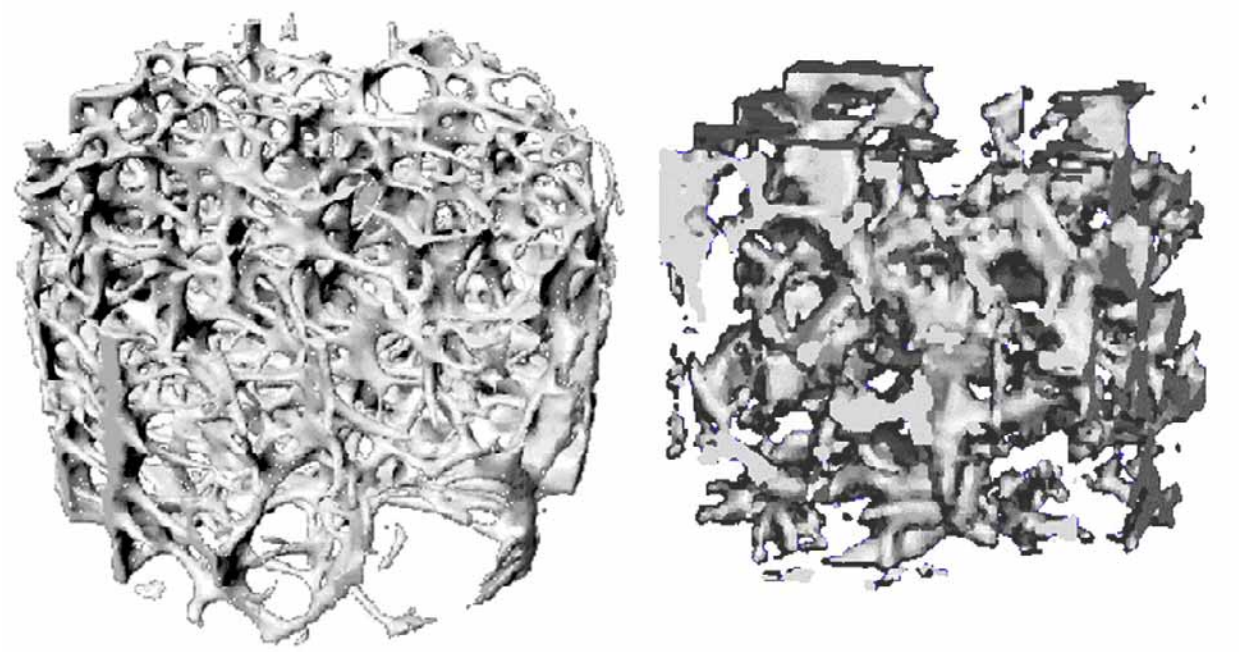

Fig. 9: Bone specimen and tissue engineered scaffold

Gas leaching involves the incorporation of a CO_2 precursor into the initiator of a polymer. The polymer is then fabricated and during initiation of the crosslinking, the CO_2 is leached from the solid creating a porosity regulated by the amount of precursor ingredients added (39). The final composition of the pore size and the total porosity can be measured by mercury porosimetry or micro computed tomography (μCT) (13, 39).

Melt molding is similar to the process of salt elution but with gelatin micro-spheres. Following the incorporation of the micro-spheres into a polymer, the mix is placed in a Teflon mold. Confined compression and heating above the glass transition temperature produces an extremely dense material. Following cooling, the gelatin particles are leached out of the material by immersion in a water bath (35). This method assumes that the polymer used in the scaffold is not destroyed at high temperatures.

There are several problems associated with these scaffold manufacturing techniques that go beyond the inability to create an engineered micro-architecture. The first problem is that pore size is often not tightly regulated. This results in grossly different mechanical properties throughout the scaffold. If this scaffold is subjected to mechanical loading, the most porous region, which generally coincides with the mechanically weakest region, will fail prematurely and cause a catastrophic failure of the implant. The apparent material properties of the scaffold therefore do not depend on the average mechanical properties of the implant but rather on the properties of the weakest region within the scaffold. This problem will arise not just with the CO_2 porogen but even after sieving has been done with NaCl and gelating micro-spheres.

The interconnectedness of the scaffold is assessed initially and is a result of the volume percent of the polymer vs. the solid particle. While the overall porosity may mimic the levels seen in bone, the structure may not be completely connected throughout the architecture. Two evaluation techniques, mercury porosimetry and μCT can be used to determine the porosity and pore size but the two techniques both have their own source of error. Porosimetry can underestimate the porosity if the scaffold is not fully connected and μCT can overestimate the porosity by including in the calculation volumes that are too small to contain cells or for fluid flow. All of the problems that arise from these techniques provide ample evidence for the usage of specific methods of scaffold fabrication.

Specific Scaffold Generation

Using methods that generate specific architecture allows for a closer approximation of the characteristics of native bone. Optimization of these procedures can result in scaffolds that contain internal architecture that is regulated with the proper porosity, density, pore size, crystallinity and diagonal length of struts. The majority of these methods have in the past been used in concert with the random methods because the current technology was unable to provide for the ability to closely regulate the internal architecture of the scaffold. These methods are fiber bonding, extrusion, and high pressure processing. The result of using these techniques is a scaffold with basic overall structure such as a cylinder or a disc (Fig. 10) (40). Extrusion has been extremely successful in the creation of scaffolds with a tubular nature. Melted polymer is extruded through a nozzle containing the desired axial geometry. Fiber bonding involves the gluing of a group of polymer fibers together using another dissolved polymer to create a global structure (35, 41). High pressure processing involves the heating of the polymer mixture above the glass temperature and then compressing it into the required shape. Following cooling, the scaffold is extremely dense and possesses high strength. Fused deposition modeling works like an inkjet printer with a movable z-stage (Fig. 11). The print heads deposit a material onto the stage in a two-dimensional pattern that is representative of a slice of the final geometry.

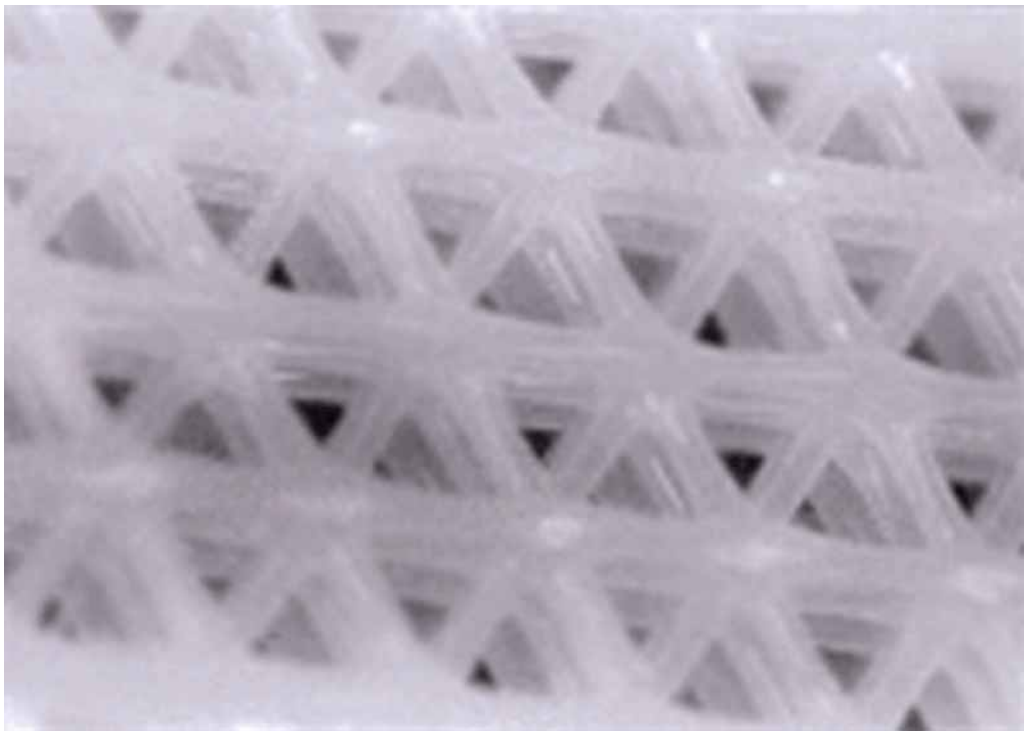

Fig. 10: Fiber Binding Technique (55)

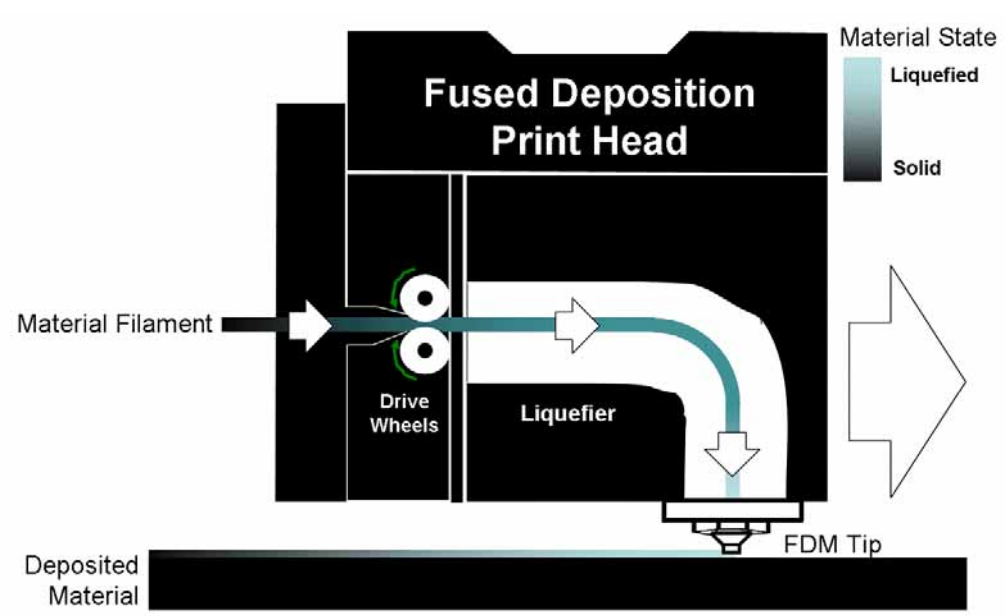

Fig. 11: Fused Deposition Modeling (53)

Rapid prototyping or solid freeform fabrication (SFF) procedures can produce regulated, engineered architecture. Several different systems exits that are able to achieve levels of magnitude based upon different design principles and methods. The most important methods of fabrication are fused deposition modeling (FDM), stereolithography, and particle binding.

Fused deposition modeling works like an inkjet printer with a movable z-stage. The print heads deposit a material onto the stage in a two-dimensional pattern that is representative of a slice of the final geometry. A secondary support material is often used to allow oblique angles to be built on slices higher than the current print layer. The final object is retrieved from the build surface by either melting or dissolution of the support material. This process can be geared to be used with several different materials, the limiting factor being the melting temperature and the cooling time upon deposition to the platform. The PatternMaster (SolidScape, Manchester, NH, USA) utilizes two thermoplastic waxes for build and support and several different solvents to remove the materials (33, 34). Thermo-reversible hydrogels have been delivered using an FDM process (24). Testing of the system indicated that the scaffolds can be built with an orthogonal structure although no support material was utilized in this process. The process has also shown promise with agarose which has an extremely low melting temperature (12). The advantage of using this technology is that the scaffolds can be cleansed easily with solvents so the support material will not be left in the small crevices of the material. Additionally, FDM is able to achieve resolution of ~100um features, which is similar to stereolithography. One disadvantage is that the fabricated object displays anisotropy in the XY plane with respect to the Z stage (34).

Stereolithography creates three-dimensional structures by photo-polymerizing a liquid polymer. A movable stage is contained in the bath of the liquid polymer and is moved down a slice following the crosslinking of one layer of material (Fig. 12). The photopolymerization may occur with the use of a masked lamp or a laser, with the laser allowing a higher resolution (34). 3D Systems offer a stereolithography machine that utilizes a UV laser to build models with a resolution down to ~40 μm feature size. Because the material is contained in a bath, the supports required in some regions can only be made of the same material, making the mechanical removal process very difficult in delicate areas. Another disadvantage of this technique is the large amount of material that is exposed to open air that may be damaged or contaminated after repeated use of the process. The

main disadvantage is the limit to the number of materials that can be used with this process although new materials are developed with the use of organic chemistry and the incorporation of photocrosslinking agents into the desired polymers. The advantage of using stereolithography is that the scaffold can be generated immediately without the required use of secondary processes to obtain the required geometry, architecture, and material composition.

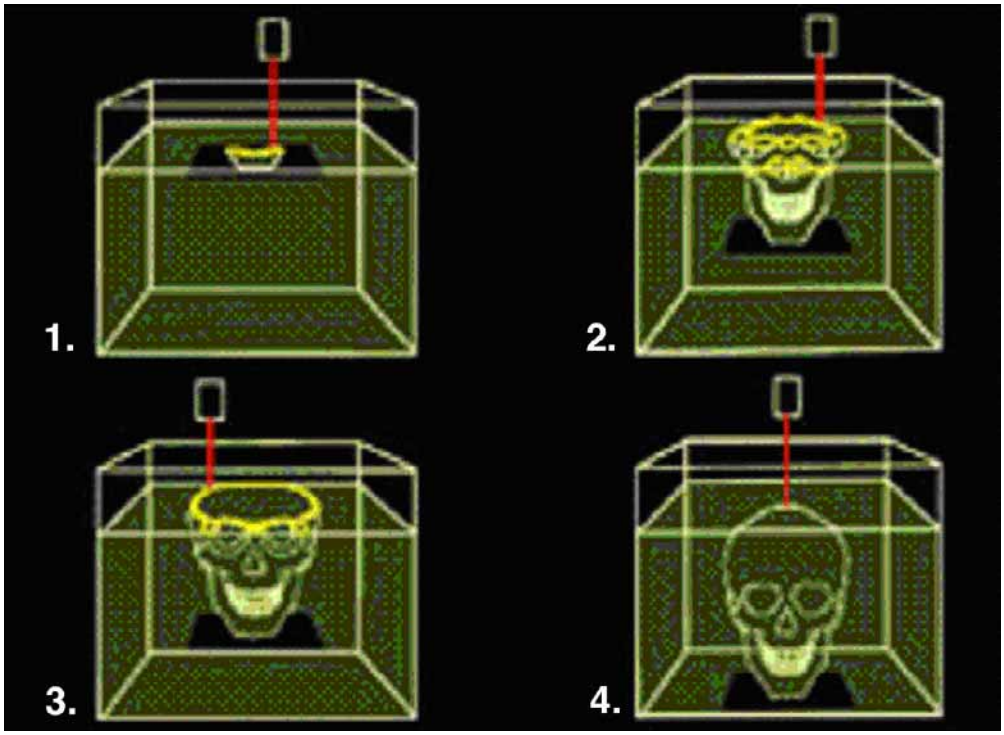

Fig. 12: Stereolithography (56)

The final method to be addressed is particle binding which creates a global structure by gluing particles together in a three-dimensional arrangement. A printhead deposits a pattern of a binder onto a bed of particles which creates a slice. The stage is then moved down and a fresh layer of particles is rolled on top of the previous level (Fig. 13). Because the object is being built in a tub of solid particles, the support is already provided and no additional materials are required. This process works with particles that can be bound together which makes it advantageous for use with ceramic particles, such as hydroxyapatite. Therics, Inc. (Princeton, NJ, USA) has developed a system that allows the incorporation of growth factors, peptides, and even drugs into the binder and subsequently the scaffold (42). The disadvantage of this technique is the low resolution that results

from an inability to achieve sharp angles or to remove additional material from small crevices. The main advantage is that this technology can produce a ready made implant that has growth factors or cells already incorporated into it.

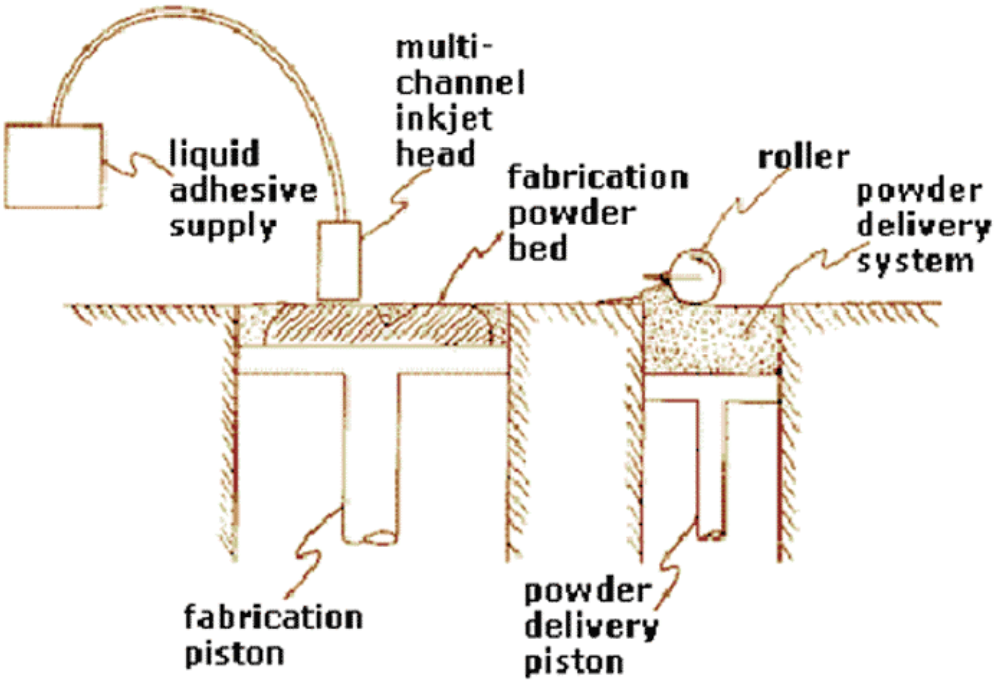

Fig. 13: Particle Binding Technique (57)

In addition to the prototyping methods just detailed, several secondary methods exist that when combined with the previously detailed techniques can create regular architecture. These are injection molding, lost wax, and +/- molding processes. The use of prototyped scaffolds with these methods in combination with other imaging techniques providing geometric boundaries will produce scaffolds that can be site and patient specific and also built with the desired material properties and resolution. When using a process such as stereolithography or FDM, the fabricated model can be used as either a positive or a negative for mold creation. This mold can then be injection molded with any material desired or can be used to generate a completely new structure. The choice of a molding material depends on the requirement of the implant. A good material to use is one that can dissolve (lost wax model) which is especially useful and required if attempting to build a scaffold

with interconnected pore structures by using mold techniques. Some of the steps from the fabled lost wax method can be used to fabricate the scaffold. If the scaffold material has a high enough melting point, the wax model can be used as a mold to hold the material and then can be melted from the scaffold.

We believe that the current methods for generating bone replacement scaffolds are just at the beginning and that the optimal design parameters still need to be defined. The architecture that can be generated by the specific methods is still not optimized based upon the hierarchy described previously. Mechanical and biological properties need to be taken into account when using either the molding techniques or with a particle binder solution. Using previous methods for the generation of the chemical level and up on the hierarchy, the architecture of the scaffolds can finally be generated to mimic bone architecture. Additionally, thanks to the incorporation of detailed micro-architectural features and understanding of the effect of the scaffold geometry on tissue ingrowth, the scaffolds can now be designed specifically to stimulate bone growth and bone modeling of a certain site.

Bone Modeling and Bone Adaptation

Previously in this review it has been stressed that the successful design of a bone scaffold for load bearing applications needs to incorporate both biological and mechanical considerations. Incorporating those theories will promote bone cells migrating into the scaffold and will enhance the natural bone modeling process. It is the current believe that bone modeling is based on mechanotransduction, the ability of the tissue to sense mechanical loading and adapt accordingly. The goal of an implant is to promote mechanotransduction through the scaffold to spurn bone modeling. Due to mechanotransduction, bone modeling will occur within the scaffold as the scaffold itself degrades. Although mechanisms of bone modeling within the actual scaffold have not yet been discovered, several theories explaining bone modeling exist based on *in vivo* observations (14).

Prevalent bone modeling theories stipulate that the primary reason for the onset of the process is as a result of dynamic strain levels that exist in bone. They further indicate that following a sustained increase in the strain energy density, bone modeling will begin. Several computer simulations and experimental evidence have shown that the modeling will occur in the dominant stress direction, with a diminished effect in the transverse directions (43). The time dependence of adaptation varies with load but usually requires a significant increase in dynamic strain for multiple cycles to affect the mechanism. In addition, recent data have indicated that high frequency loading may also stimulate bone growth (44). The apposition rate during modeling is with 2-10 μm/day relatively fast compared to the rate at which the general bone remodeling occurs (0.3-1 μm/day) (45). One of the dominant theories describing the bone adaptation process is Frost's Mechanostat Theory. Frost has defined the strain levels for which bone will be modeled and termed these strain level as the minimum effective strain (MES) (46). Fig. 14 indicates the effects of various values for the MES, below which the optimal value resorption without deposition will occur and above which necrosis will set in.

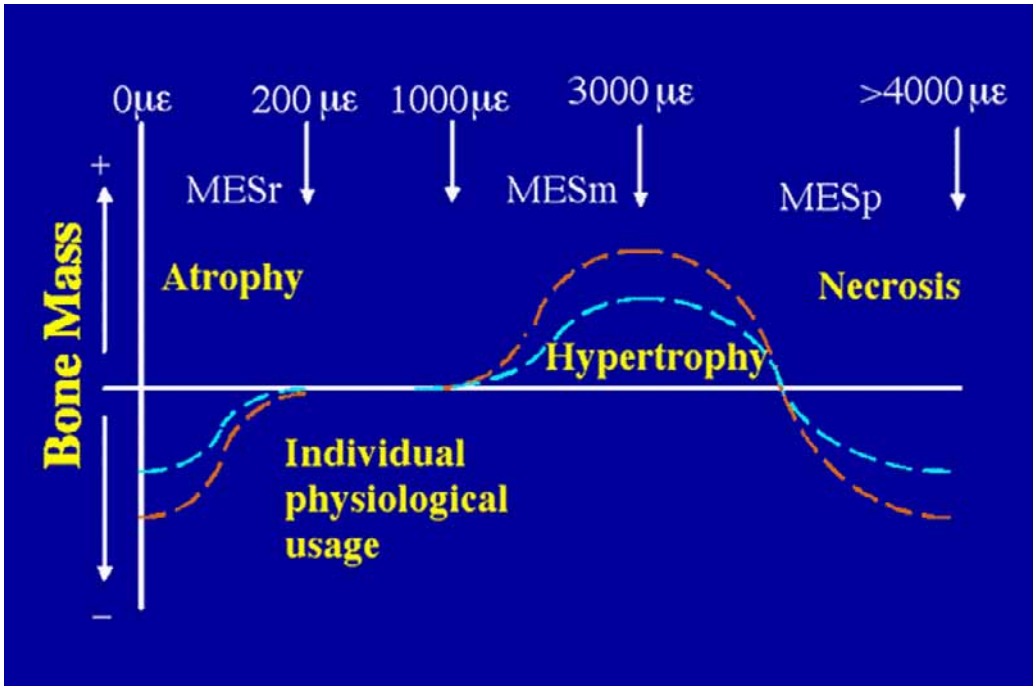

Fig. 14: MES vs. Bone Mass

Following strain sensing by the mechanosensing bone cells (osteocytes) located in the lacunae, the modeling process is initiated. The sign of the strain (positive or negative) will determine whether bone is resorbed or deposited. Negative strain levels will result in osteoclast recruitment which will begin the resorption of bone using a complex combination of enzymes and low pH environment. The reduction in bone will continue until an equilibrium strain level is reached after which bone resorption will cease. If the strain is positive, osteoblasts are recruited and begin to deposit new lamellar bone. Following the recruitment signal for osteoblasts, they attach to the surface and begin to create Alkaline Phosphatase (ALP) in high amounts. This enzyme has been shown to play a pivotal role in the mineralization of the deposited collagen fibers. These processes take place on the surface of the individual tissue and are a result of the signaling of many osteocytes, representing a large strain difference over a surface. This process usually takes just over two weeks but can take much longer in disorders affecting bone growth (45). The limits of this process are that bone will only deposit on the surface, which indicates the need for a high surface/volume ratio of the scaffold similar to trabecular bone (Fig. 15).

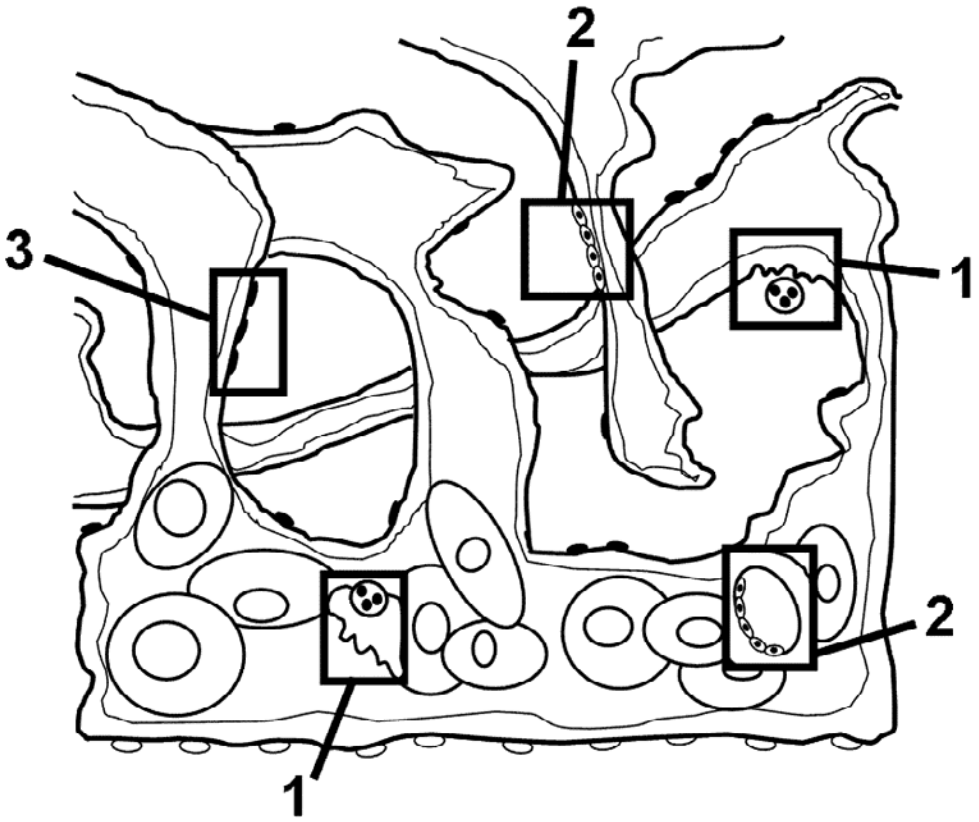

Fig. 15: Bone Remodeling Principles

Computer simulations have recently been used to predict bone growth using several of the dominant modeling schemes, such as Frost's Mechanostat, or Cowin's theory of stress modeling (46, 47). These bone modeling simulations have attempted to approximate bone growth through simulation of bone as an organ. Models such as these may help to elucidate the yet to be defined mechanism of bone modeling and may aid in the testing of implants for bone modeling on their surface. Such programs may also aid in the optimization of scaffolds for the 5 levels of bone hierarchy previously discussed. The basis for these modeling programs is finite element analysis (FEA) of bone sections obtained from μCT scans of bone volumes. In a simple two-dimensional study performed by Huiskes and associates (43), a model of bone architecture was created that was then subjected to stress loading in several directions. The bone remodeling rules that were set for this simulation were based upon strain sensing and adaptation in bone. The simulation indicated that bone remodeling occurs in the direction of the dominant stress concentration (43). The same modeling scheme was recently completed in three-dimensions with the same rules as for the two-dimensional study (48). Results verified the earlier model and it was shown that stress in only one axis resulted in pillars of bone following remodeling. The indications from the bone modeling studies show that simple bone morphology can result in a complex structure. The finite element method may be used to construct a simple morphology that can be used in these scaffolds. Additional studies have been completed using cellular autonoma theory as the basic rule for remodeling. Cellular automata are discrete dynamical systems, consisting of many identical elements interacting according to simple rules, together resulting in a complex behavior. These studies can be used for basic scaffold or material reordering based on the principles of strain remodeling (49).

Conclusion

Bone is a dynamic system that requires constant mechanical stimulation to maintain its properties and shape. Scaffolds designed for repair of bone defects in load bearing sites need to be designed for these mechanical requirements. While it is important for bone to be integrated in these defects through the scaffold, it is of major importance that high quality bone is built, which in the long run can function as the normal tissue did before the defect occurred. Just because engineered bone looks like bone does not mean that it will perform adequately under mechanical loading. For this reason, scaffold testing needs to be accomplished under simulated loading or in an implant site that experiences mechanical stress.

The goal of scaffold design for load bearing applications is to regrow bone in the defect site that is of high quality, in that it performs biomechanically adequately, and has a remodeling rate similar to that of the surrounding tissue. This review attempted to define an approach for the design of scaffolds for the intended use of healing bone defects at load bearing sites. The most important message to be taken from this chapter is that the final geometry, architecture, and mechanical properties of the bone will be completely different from that of the implanted scaffold. Because of this, the scaffold needs to be designed with mechanical and biological factors in mind. The mechanical factors are responsible for providing the structural stiffness and strength to sustain the mechanical loading, while the biological factors promote tissue ingrowth, vascularization and nutrient supply. This chapter further elucidated the issue that optimization of scaffolds based on the mechanical and biological factors needs to occur at several hierarchical levels of bone. Due to this discussion of the hierarchical levels of bone, scaffold fabrication methods, and bone modeling response, a paradigm shift in the basic set of rules for scaffold generation emerged. We are just at the beginning of understanding all the different parameters, which play a role in designing of an effective scaffold. New manufacturing techniques as described here give us the tools to investigate them and determine their importance. Following these suggested rules may lead to the design of a scaffold that more closely emulates the properties of bone and may successfully heal critical size bone defects in load-bearing applications.

Acknowledgements

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List of Figures and Captions

Fig. 1: Bone Hierarchy. This cartoon depicts several of the levels of the hierarchy that makes up bone. **A**: The top level is the whole bone structure which is shown with the femur. **B**: The next lower level is the structural or architectural level which is shown by the osteons in cortical bone and the rods and plates for trabecular bone. **C**: The drawing depicts the next level, the tissue level, and shows one separate osteon and a single trabeculae. Within both of these structures lay the lacunae with the encapsulated osteocytes.

Fig. 2: Human Femur. The femur is an example of the whole bone level. The bone has a geometry that interacts with the adjacent bones as well as the hip socket.

Fig. 3: Trabecular bone picture (50). A scanning electronic micrograph showing the architecture of trabecular bone that consists of rods and plates and is found as occurs in long bones.

Fig. 4: Cortical bone (15). This picture shows a section of cortical bone. The arrow is highlighting the encapsulated osteocytes that are part of the the laminated structure of the osteons. The lacunae lie at the center of the osteons and are the centers of remodeling in cortical bone as well as the path for nutrients and blood to flow through.

Fig. 5: Lamellae picture (15). In this composite picture of both cortical and cancellous bone, the lamellae can be seen clearly in the diagram. The difference between lamellae of cortical bone and lamellae of trabecular bone can also be seen.

Fig. 6: Pre and post-operative patient with microtia (16). The patient suffered from microtia on the right side of the head. The picture on the left shows the structure of the ear prior to treatment. The picture on the right illustrates the success of the cosmetic treatment in building the structure of an ear.

Fig. 7: Coastal cartilage shaped into ear structure (16). These two pictures illustrate both the initial and final shape and geometry. The coastal cartilage in the left panel was obtained from the patient's rib area in a surgical pretreatment. The picture on the right shows the final shape of the cartilage after it has been reformed into the shape of the ear.

Fig. 8: Composite orthogonal structure. This photogragh is a scaffold composed of two materials containing a structure with orthogonal beams. The top of the scaffold was fabricated with Poly(lactic acid) and the bottom of the scaffold is made of Hydroxyapatite. This scaffold was created using Fused Deposition Modeling combined with mold processing.

Fig. 9: Bone specimen and tissue engineered scaffold. The picture on the left shows a 5 mm^3 section of human vertebral trabecular bone. The picture on the right shows a 3 mm^3 section of a PLGA scaffold manufactured using the solvent casting technique with NaCl cristals.

Fig. 10: Fiber Binding Technique. Fibers are extruded through a nozzle and bonded together. Regular geometric shapes can be manufactured that way, however the features are limited to arrangement of the fibers within a two-dimensional plane (55).

Fig. 11: Fused Deposition Modeling. This figure illustrates the process of generation of one of the matrix levels. The extrusion of the filament through the nozzle which is heated melts the material and allows it to pass through the nozzle and be deposited to the surface of the build plate. After the material on the build plate is cool, the plate moves down to build the next level of the structure (54).

Fig. 12: Stereolithography. This drawing illustrates the process of stereolithography. (1) The material is in a bath with a submerged stage. A laser (red) photocrosslinks the material in the bath in a specific pattern. (2 and 3) Following crosslinking, the stage moves down to allow the crosslinking of an additional layer. (4) Layer by layer, the skull is built (56).

Fig. 13: Particle Binding Technique. The working principle of a particle binding rapid prototyping machine. The tray on the left contains powder, which is transferred to the right tray using the roller.

The powder is then binded with an adhesive in the pattern of the part to be made. Once a layer is finished the roller adds another layer of power and the process is repeated (57).

Fig. 14: Minimum effective strain (MES) vs. bone mass. This drawing shows the results of Minimum effective strain on the modeling process of bone. At the low levels, bone is resorbed from the body. There exists a median range where bone is added but this tops off at an optimum value. High values for strain will cause tissue to necrose and will result in an overall loss of bone.

Fig. 15: Bone Remodeling Principles. Both cortical and trabecular bone are constantly remodeled. Initially, bone is resorbed by osteoclasts both in the cortex and on the trabeculae (1). Bone formation by means of osteoblastic activity occurs on the site of the resorbed bone (2). The osteoblasts themselves become incorporated into the bone as osteocytes (3).